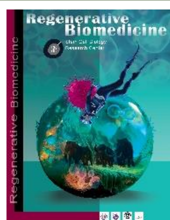

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Review Article

Appropriate Biomaterials for Scaffold Fabrication to Induce In Vitro Spermatogenesis

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Abstract

The contribution of male variables to cases of infertility is believed to be between 30% and 50%. In vitro spermatogenesis and male fertility preservation can be achieved by mimicking testicular natural extracellular matrices and 3-dimensional (3D) cell culture. In recent years, tissue engineering (TE) has employed novel methods for the preservation of male fertility by generating functional male reproductive cells. Scaffolds, a crucial element of TE, are created using natural or synthetic biomaterials, or a combination of both. The utilization of natural material-based scaffolds can significantly enhance the efficiency and improve in vitro spermatogenesis compared to synthetic material-based scaffolds. Only a few investigations that have focused on in vitro spermatogenesis have yielded significant quantities of mature gametes to date. Original and review articles were obtained by searching the PubMed and Google Scholar databases. The present review focuses on the natural and synthetic materials used in the process of in vitro spermatogenesis for the purpose of fertility preservation.

Keywords: Biomaterials, In-vitro spermatogenesis, Three-dimensional culture, Tissue engineering, Scaffold

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Introduction

The failure of a couple to conceive after a year of trying is known as clinical infertility. It is estimated that male factors are responsible for 30–50% of infertility cases. Testicular dysfunction, congenital anatomical abnormalities, lifestyle variables (such as obesity and smoking), endocrinopathies, gonadotoxic exposures, and aging can all lead to infertility or diminished fertility (1). Tissue engineering (TE) has advanced novel methods for preserving male fertility by developing cells known as male germ cells. This suggests that TE may be a viable approach for the future restoration of male fertility (2). Combining the use of cells, scaffolds, and bioactive molecules is sometimes referred to as a tissue engineering triad (3). Cells are one essential element of TE. In addition, various kinds of cells can be employed in the field of testicular tissue engineering. Mixtures of testicular cells, purified spermatogonial stem cells (SSCs) populations, and nontesticular cell types, including mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), epiblast stem cells (EpiSCs) and embryonic stem cells (ESCs), have been employed for generating sperm in vitro (4-9). Another contributing factor to TE is growth factors (GFs). GFs are soluble polypeptides that play a crucial role in controlling the survival, migration, proliferation, and differentiation of cells. A unique combination of factors, including EGF, LIF, GDNF, bFGF, estradiol, and progesterone, is introduced to the culture medium of in vitro spermatogenesis to enhance proliferation and induce differentiation of spermatogonial stem cells (10). The scaffolds are the final element in TE. According to earlier research, scaffolds made of both natural and synthetic polymers have been applied to testicular tissue engineering (11-13). Due to the composition of the extracellular matrix (ECM), which consists mostly of proteins and polysaccharides, two categories of natural polymers, namely proteins (such as collagen) and polysaccharides (such as chitosan and alginate), have been employed to organize testicular cells in a specific manner (14). So far, several types of natural and synthetic materials have been used to study spermatogenesis. The objective of this study is to investigate suitable biomaterials for scaffold fabrication to induce in vitro spermatogenesis.

Natural biomaterials

Collagens are the most extensively researched polymer of biological origin, as they are the most prevalent structural protein in the extracellular matrix. Collagen-based hydrogels have provided a niche for testicular cell reaggregation and established a favorable environment for germ cell culture, differentiation, and maturity. Zhang et al. observed that the culture system promoted the differentiation of spermatogonia into primary spermatocytes by culturing neonatal mouse testicular cells on collagen-based hydrogel with Knockout Serum Replacement (KSR). The blood-testis barrier and structures like seminiferous tubules were also developed (15). In a separate investigation, rat testicular cells effectively developed colonies on the collagen scaffold for a duration of 35 days and commenced the formation of tubules. Nevertheless, there were no haploid cells seen (16). Gelatin is a non-immunogenic, inexpensive alternative to

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collagen that is produced by the physical, chemical, or thermal hydrolysis of collagen. Additionally, it has the capability to promote the attachment, proliferation, and differentiation of many kinds of cells. In order to be more similar to the ECM of natural tissue, gelatin can be electrospun to prepare an artificial fibrous structure. The impact of electrospun gelatin (EG) scaffolds on the attachment and proliferation of sertoli cells and ESCs was investigated by Vardiani et al. The study's findings indicated that the scaffold was neither cytotoxic to sertoli nor ESCs. Furthermore, ESCs and sertoli developed and adhered well to EG (12). Combining collagen gel with Matrigel or using it alone has made it possible for germ cells to be close to somatic cells and the extracellular matrix. In one investigation, collagen gel (CG) or collagen plus matrigel (CGM) was used to cultivate rat testicular cells. These scaffolds had the capacity to reorganize isolated cells and create a cyst-like structure that includes Sertoli and Leydig cells. Moreover, the meiotic and post-meiotic development of germ cells were supported (17). It is important to note that matrigel can also be used to facilitate the development of in vitro male reproductive cells without the need for a combination of other natural or synthetic-based polymers. Human SSCs were cultivated on a 3D system made of Matrigel under specific conditions. This culture technique allows the SSCs to differentiate and produce functional haploid spermatids. The differentiation efficacy of this culture system is estimated to be as high as 17.9% (18). Alginate is an additional biomaterial that is readily accessible and has a broad range of applications, including cryopreservation, cell immobilization, and 3D cell culture. The biocompatibility, oxygen diffusivity, hydrophilicity, nutrient release, and antioxidant activity of alginate hydrogels have the potential to improve cell survival and facilitate their proliferation. Jalayeri et al. seeded newborn mouse SSCs within the alginate hydrogel. The assessment of cytotoxicity in the hydrogel-containing cells indicated that alginate hydrogel is suitable for in vitro cultivation of SSCs due to its antioxidant capabilities. However, this study did not evaluate the development of spermatogenesis (19). In 2019, Yoni Baert et al. fabricated 3D bio-printed scaffolds based on alginate to regulate the design of the scaffold and the placement of cells. The results of this investigation demonstrated that tubular structures were not formed, but cell spheres were obtained after one week. Another finding was the presence of postmiotic cells; however, full spermatogenesis did not occur (20). A further potential candidate for the formation of scaffolds is chitosan. This polycationic biopolymer is typically derived from chitin by alkaline deacetylation. Chitosan has garnered significant interest in the field of biological applications due to its biodegradability, biocompatibility, minimal immunogenicity, and antibacterial ability (21). In a study, a bioreactor composed of a hollow cylinder of chitosan hydrogel was used to cultivate segments of fresh and frozen seminiferous tubules from immature rats and adult humans for a period of 60 days. It led to mature spermatozoa that were morphologically normal. Furthermore, haploid cells were identified in human cultures in a range of 2% to 3.8% (11). Acellular tissue matrices that are produced by decellularizing various tissues or organs can

maintain the essential components and growth factors of the ECM and their functions to a satisfactory degree, therefore ensuring the integrity required for cell proliferation (22). Recent decellularization protocols have been suggested in accordance with the tissue type, tissue density, and biological characteristics, and they include chemical, physical, and enzymatic methods (23, 24). Optimization of testicular tissue decellularization procedures has been conducted to maximize cell proliferation and ECM structure and composition (23). Decellularized testicular tissue-derived scaffolds have been made in the form of whole organs, plates, fragments, hydrogel, testis-derived macroporous scaffolds and 3D bio-printed scaffolds for in vitro spermatogenesis (4, 10, 25-27). Sheep testicular tissue-derived scaffolds were fabricated by Tohid Rezaei et al. In this study, the incubation of neonatal mouse testicular cells on the mentioned scaffolds led to the production of organoids, in which the differentiation of spermatogonial cells into post-meiotic cells was confirmed, although their efficiency was low and no sperm were generated. In addition, hormonal analysis demonstrated the role of testicular organoids in the secretion of inhibin B and testosterone. In this investigation, it was proposed that these scaffolds could serve as a novel substrate for in vitro spermatogenesis and testicular tissue engineering (4). Naeemi et al. (2021) seeded SSCs of neonate mice into a scaffold composed of decellularized mouse testicular matrix, chitosan, and hyaluronic acid. The investigation revealed that the engineered scaffold was non-toxic to cells, and the presence of PLZF, TP1, and TEKT1 markers validated the ability of SSC to proliferate and differentiate on this scaffold. Consequently, the fabricated scaffold may serve as a layer of support for the proliferation and differentiation of cells (28).

Synthetic materials

Suitable scaffolds for testicular tissue engineering have been constructed using synthetic materials in addition to natural biomaterials (29, 30). For biomedical applications, polyvinyl alcohol (PVA) is regarded as one of the most preferable polymers due to its biodegradability, biocompatibility, non-toxic properties, and low cost (31). Kashani et al. (2020) reported that the use of PVA in combination with agar (agar/PVA electrospun nanofibers) has the ability to enhance the rate of differentiation of mouse SSCs into meiotic and post-meiotic cells (30). A scaffold was constructed using poly (D, L-lactic-co-glycolic acid) or PLGA in a separate study. This was followed by the seeding of immature rat testicular cells onto the scaffold's surface. This scaffold promoted spermatogenic germ cell proliferation and differentiation more than conventional or organ culture (13).

Material and method

On the basis of the PRISMA 2020 guidelines, the present narrative review is conducted. An extensive literature review was done on the application of appropriate biomaterials in testicular tissue engineering as a possible therapy for male infertility. The keywords 'scaffold', 'tissue engineering', 'in vitro spermatogenesis','three-dimensional culture', and 'biomaterials', in addition to their associated equivalents in Mesh, were searched articles are included in the

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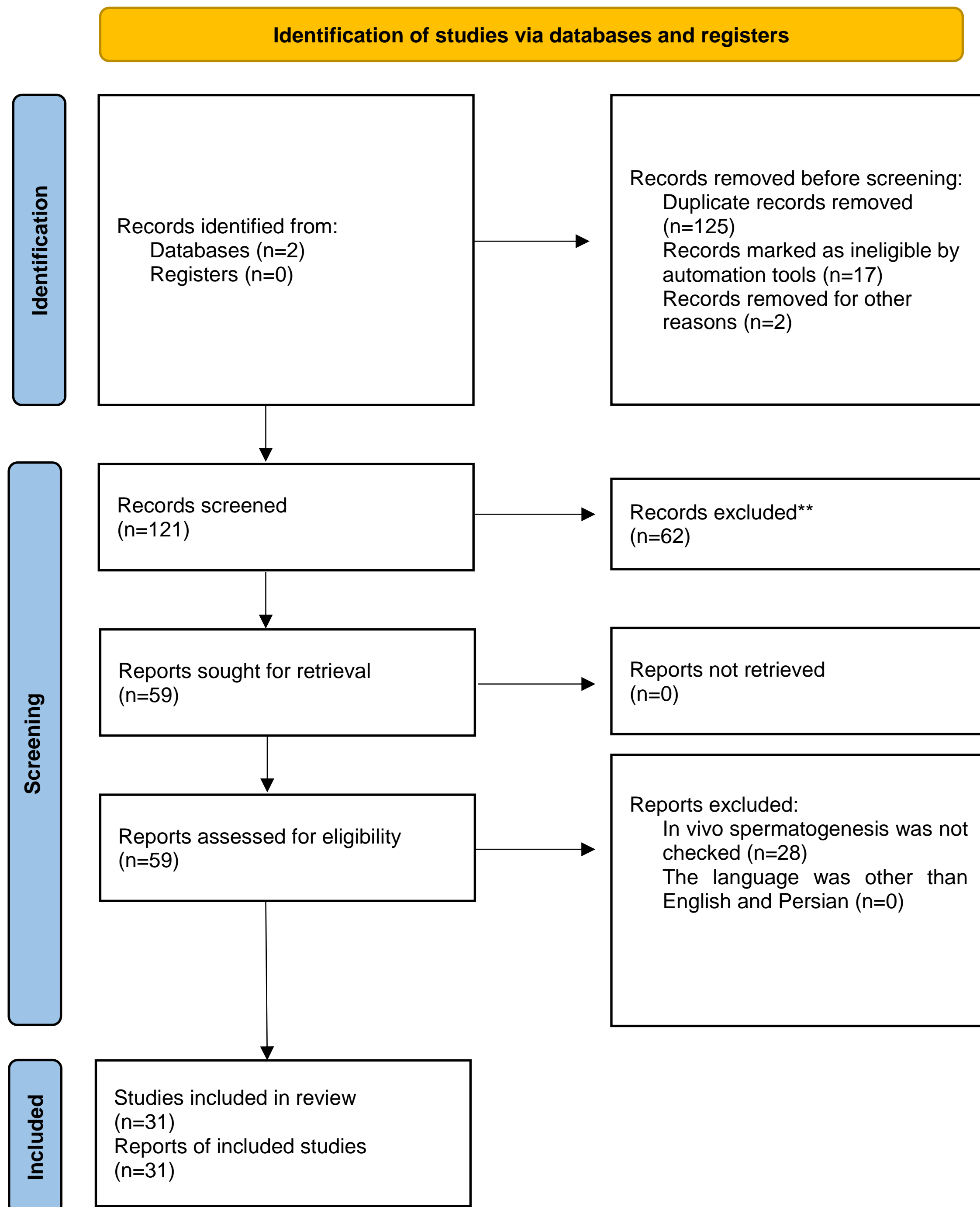

Figure 1. Study selection diagram for narrative review according to PRISMA 2020 guidelines. The diagram displays the quantity of search databases. Also, the numbers and types of screened and excluded studies were investigated.

investigation. A narrative review diagram from PRISMA 2020 was utilized to illustrate the choice of articles (Fig. 1).

Conclusion

Because of the rising prevalence of male infertility, there is a greater demand for new

artificial testicular systems to maintain fertility. For the normal function of sperm, an effective culture system will be required, as the altered genetic material generated by haploid germ cells during in vitro spermatogenesis will be transferred to the offspring. In comparison to synthetic material-based scaffolds, the efficacy of in vitro spermatogenesis can be substantially improved through the use of natural material-based scaffolds. Despite incomplete differentiation, the 3D matrix of decellularized testicular tissue seems to facilitate the formation of organoids by testicular cells. Moreover, mature spermatozoa of rats and humans were obtained by long-term culture of seminiferous tubules in a chitosan-based bioreactor. Considering the limitations and positive features of biomaterials in specific aspects, composite scaffolds can be a good choice for in vitro spermatogenesis studies. Scaffolds derived from decellularized testicular tissue and biocompatible natural polymers, such as chitosan, may be used for in vitro spermatogenesis..

Conflict of interest

The authors have no conflict of interest to declare.

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